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Full Length Research Paper

A binding of ECM proteins - collagen, fibronectin, albumin and vitronectin by *Bacteriocinogennic enterococci* isolated from chicken and rabbits

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Ten *Bacteriocinogennic enterococci*, isolates from chicken and rabbits, were examined for their binding of collagen, fibronectin, albumin and vitronectin in tubes with Nutrient broth No.2. For ECM binding examination, enterococci were grown in MRS broth under normal air conditions at 37°C. Individual strains expressed binding of selected glycoproteins to various degrees, however, vitronectin was bound the best from these four ECM proteins. These observations lead to suggestion that at least one mode of enterococcal attachment utilizes glycosaminoglycan chains present on the surface of adherent cells. Significance and impact of the study is that some animal strains are comparable with the commercially used strains with respect to their ECM ability. As this feature is important for probiotic bacteria to be able to colonize intestine, these strains could be considered for their wider use in fermented feed for animals.

Key words: *Enterococcus*, extracellular matrix, collagen, fibronectin, albumin and vitronectin.

INTRODUCTION

During the past two decades probiotic microorganisms have been increasingly included in various types of food products, especially in fermented milks (Saarela et al., 2000). However, there is also an increasing scientific and commercial interest in using beneficial microorganisms to enhance animal health and feed conversion (Chang et al., 2001; Francisco et al., 1995). Many selection criteria have been considered to be relevant for any potential useful microorganism. Sucessful bacteria should be able to colonize the mucosal surfaces, at least temporarily and to prevent the attachment of pathogens such as *Escherichia coli* (Lee et al., 2000) and other intestinal or food/borne pathogens (Todoriki et al., 2001). Molecules of the ECM such as collagen and fibronectin can be shed into the mucus from the epithelium. Damaged host mucosae expose the ECM and this allows microbial colonization and infection. Moreover, ECM binding ability has been shown to be expressed by several pathogenic bacteria and to promote bacterial virulence (Lowrance et al., 1990; Hienz et al., 1996). Selected bacterial strains should be able to compete with pathogens for the same receptors and to occupy their potential binding sites in the gut (Neeser et al., 2000) including collagen, fibronectin (Lorca et al., 2002) and other ECM proteins. The ability of bacteria to produce bacteriocins is well known (Klaenhammer, 1993). Bacteriocins are proteinaceous compounds with inhibitory activity against more or less related bacterial genera (Nes and Holo, 2000). Strains of enterococci, especially the specium of *Enterococcus faecium* possess also the ability to produce bacteriocins (Lauková et al., 1993; Strompfová et al., 2003). Enterococci selected for our study represent bacteriocinogennic strains, described previously by Strompfová (2004) and Simonová (2006). Because of their use and other characterization, they were investigated for binding of four selected glycoproteins which are often found also in intestinal part of organism.

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Abbreviation: ECM, Extracellular matrix.

Table 1. The absorbance A $_{570nm}$ difference after proteins sorbcion effect to bacteriocin producing bacterial cells.

Chemical concentration	Fibronectin (g/l)		Vitronectin (g/l)		Collagen (g/l)		Albumin (g/l)	
Bacterial kind	0.01	0.005	0.1	0.5	10	50	1000	5000
EF 55	+	+	+++	+++	+	+	-	-
EFH 31	-	-	+++	+++	+	++	-	+
EF2S3	-	-	+++	+++	-	-	-	+
EF 1839	-	-	+++	+++	-	-	-	-
EF 529	++	++	+++	+++	++	+	+	+++
EF 24/10	-	-	+++	++++	-	++	+	++
EF 2019	-	-	+++	++++	-	-	-	+

Legend: (+) 0.01 – 0.02, (++) 0.02 – 0.04, (+++) 0.04 – 0.06, (++++) 0.06 – 0.08.

MATERIALS AND METHODS

Ten (10) enterococcal isolates from our collection (IAP, Košice, Slovakia) were investigated. These strains were originally isolated from poultry and rabbits. For ECM binding examination, enterococci were grown in MRS broth (Merck, Germany) under normal air conditions at 37°C for 18 h. Then were compared bacteriocin-producing strains with non-producing strains. Seven isolates are able to produce bacteriocin-like substances towards more or less related Gram-positive bacterial genera (e.g. Enterococcus, Staphylococcus, Listeria spp.) and are possessing two or three genes for production of bacteriocins /A, P, or L50B/ as described earlier (Simonová and Lauková, 2007).

Chemicals

Fibronectin, collagen, albumin and vitronectin were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). MRS broth was purchased from Merck (Germany).

Protein binding assay

Tubes were filled with MRS broth and inoculated with individual strains. Subsequently they were incubated overnight at 37°C and after growth of the strains, individual proteins were added to tubes. After 2 h of incubation at room temperature, A_{570nm} readings were written. These A_{570nm} readings were obtained by measuring on UV-VIS Spectrometer UVG 124622, type Helios Gamma 100 - 240 (England). Each batch of assays also included control which was compared to these obtained values. Bacteria were classified as strongly adherent, weakly adherent or nonadherent as described by Štyriak et al. (1999). This method was described in detail earlier by Štyriak et al. (1999).

RESULTS

Individual strains expressed binding of selected glycoproteins to various degrees as shown in Table 1. It is visible in this table that vitronectin was bound better than collagen and fibronectin, however, albumin was bound very good especially by strain EF529. Concerning the characteristics about genes and bacteriocin-like substances, we would like to mention previous publications of Strompfová et al. (2003) and Simonová and Lauková (1993).

The pre-incubation of selected enterococcal strains with heparin for 2 h at room temperature decreased their binding reaction with ECM proteins. This observation shows that at least one mode of enterococcal attachment utilizes glycosaminoglycan chains present on the surface of adherent cells. This suggestion was confirmed by subsequent PAA (particle agglutination assay) when most of our isolates bound heparin (data not shown). No difference between bacteriocin producing and non-producing strains (it means without this ability) was observed. It means bacteriocin production has no influence on binding of these strains to selected glycoproteins.

DISCUSSION

Since enterococci are commensal organisms and part of the normal flora of most humans and animals, their virulence factors are typically subtle. The mechanisms by which enterococci progress from a state of commensal colonization to cause infection are unknown. Bacteriocin production is considered also as factor influencing colonization or invasive infection in Enterococcus species. However, several factors have been implicated as potential virulence traits. Enterococci can readily acquire, accumulate and share extrachromosomal elements encoding virulence traits or antibiotic resistance genes. This is an advantage for their survival under environmental stress conditions (Jett et al., 1994). Therefore, enterococci can act not only as important nosocomial pathogens but they may complicate antibiotic therapy in veterinary medicine as well as in people working in animal weaning because of possible transfer between animals and humans. This fact is supported by the study of van den Bogaard et al. (1997) which shows that humans and animals in close contact could harbour the same vancomycin-resistant strains.

As written earlier, the next step of our study is directed to a comparison of the binding of selected ECM glycoproteins by our enterococcal collection. Our study demonstrates that many animals excrete multiresistant

Table 2. The absorbance A $_{570nm}$ difference after proteins sorbtion effect to bacteriocin non-producing bacterial cells.

Chemical concentration	Fibronectin (g/l)		Vitronectin (g/l)		Collagen (g/l)		Albumin (g/l)	
Bacterial kind	0.01	0.005	0.1	0.5	10	50	1000	5000
EF 9296	-	+	+++	+++	+	+	+	+
SX 310	-	-	+++	++++	-	-	-	-
EF 259	-	-	+++	++++	+	+	-	-
EF 819	-	+	+++	++++	-	-	-	+
EF 929	-	-	+++	++++	+	-	+	+

Legend: (+) 0.01 – 0.02, (++) 0.02 – 0.04, (+++) 0.04 – 0.06, (++++) 0.06 – 0.08.

enterococci in faeces, able to bind several glycoproteins. This may be a reservoir for those bacteria which can also be found in several kinds of feed Table 2.

Virulence traits include adherence to host tissue, invasion and abscess formation, modulation of host inflammatory responses and secretion of toxic products (Eaton and Gasson, 2001). That is why the occurrence of known potential virulence factors of enterococci such as hemolysin-cytolysin, gelatinase, enterococcal surface protein and aggregation substance (Xu et al., 1997; Archimbaud et al., 2002) has often been determined. For enterococci, to act as pathogens, they must first adhere to host tissues. Enterococci express factors that permit adherence to host cells and extracellular matrix (Jett et al., 1994). We have also shown in previous reports that enterococcal isolates from humans (Zareba et al., 1997), farm animals and dung (Štyriak et al., 1999) as well as wild herbivores (Štyriak et al., 2002) bind individual ECM components to various degrees. Now, we extend our study by screening of selected isolates from animals for their binding of some ECM molecules serving also as potential receptors for bacteria. Our isolates originated from healthy animals without any clinical manifestations of an illness. Therefore, this can be a reason of their lower binding activity of ECM glycoproteins. Moreover, there is also necessary to consider possible masking of some receptors by other bacterial surface structures as suggested in the previous report of Zareba et al. (1997).

Since the aim of our study was to ascertain enterococcal attachment to extracellular matrix, we subsequently decided to investigate an inhibitory effect of glycosaminoglycans and mucin on enterococcal binding. Glycosaminoglycans (GAGs) are found ubiquitously in the human and animal body and form a major part of the extracellular matrix (Wadström and Ljungh, 1999). Heparin (a heavily sulphated GAG with negatively charged structures and remarkable propensity for protein binding) is released by mast cells in an allergical response. Heparin binding was confirmed also in most of our isolates by PAA. Since our experiments suggest that also animal enterococci utilise this mode of ECM attachment, subsequent research in this field should be interesting also for veterinary community.

In conclusion, bacteriocins produced by intestinal Enterococcus isolates may help to control the autochthonous microflora and may be advantageous to the producing strains for their establishment and competition in the gastrointestinal tract (Strompfová et al., 2003). These bacterial strains could be used in animal feed to prevent colonization by harmful bacterial strains. By the way, vitronectin is a 75-kDa adhesive glycoprotein that also serves to promote the attachment and spreading of a wide variety of cell types to culture plastic and cell-bacteria adherence.

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